Effect of Sanitizer Treatments on *Salmonella* Stanley Attached to the Surface of Cantaloupe and Cell Transfer to Fresh-Cut Tissues during Cutting Practices†

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MS 00-380: Received 25 October 2000/Accepted 12 March 2001

ABSTRACT

The ability of *Salmonella* Stanley to attach and survive on cantaloupe surfaces, its in vivo response to chlorine or hydrogen peroxide treatments, and subsequent transfer to the interior tissue during cutting was investigated. Cantaloupes were immersed in an inoculum containing *Salmonella* Stanley (10^8 CFU/ml) for 10 min and then stored at 4 or 20°C for up to 5 days. Periodically, the inoculated melons were washed with chlorine (1,000 ppm) or hydrogen peroxide (5%), and fresh-cut tissues were prepared. The incidence of *Salmonella* Stanley transfer from the rinds to the fresh-cut tissues during cutting practices was determined. A population of 3.8 $\log_{10}$ CFU/cm^2 of *Salmonella* Stanley was recovered from the inoculated rinds. No significant ($P < 0.05$) reduction of the attached *Salmonella* population was observed on cantaloupe surfaces stored at 4 or 20°C for up to 5 days, and the population was not reduced after washing with water. *Salmonella* Stanley was recovered in fresh-cut pieces prepared from inoculated whole cantaloupes with no sanitizer treatment. Washing with chlorine or hydrogen peroxide solutions was most effective immediately after inoculation, resulting in an approximate 3.0-$\log_{10}$ CFU/cm^2 reduction, and the level of recovered *Salmonella* population transferred to fresh-cut samples was reduced to below detection. The effectiveness of both treatments diminished when inoculated cantaloupes stored at 4 or 20°C for more than 3 days were analyzed, and the fresh-cut pieces prepared from such melons were *Salmonella* positive. *Salmonella* outgrowth occurred on inoculated fresh-cut cubes stored above 4°C.

Fruits and vegetables are frequently in contact with soil, insects, animals, or humans during growing or harvesting *(18)* and in the processing plant. Thus, their surfaces are exposed to natural contaminants, and by the time they reach the packinghouse, most fresh produce retains populations of 10^4 to 10^6 microorganisms/g *(2, 3)*. There are many reports of disease due to consumption of fruits and vegetables that were contaminated at the surface with enteric pathogens *(2, 9)*. The safety of fresh and fresh-cut produce available in salad-bar operations and supermarkets is a concern *(12)*.

Salmonellosis has been steadily increasing as a public health problem in the United States since reporting began in 1943 *(19)*. *Salmonella* is among the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States *(15)*. Three multistate outbreaks of salmonellosis have been associated epidemiologically with cantaloupes. The first involved *Salmonella* Chester, which affected 245 individuals (two deaths) in 30 states *(16)*. The second involved more than 400 laboratory-confirmed *Salmonella* Poona infections and occurred in 23 states and Canada *(4)*. The most recent (April to May 2000) outbreak was due to *Salmonella* Poona associated with 43 illnesses *(7)*. These outbreaks were associated with the consumption of cantaloupe from salad bars or in fruit salads.

Salmonella may have survived on the cantaloupe surfaces following contamination at the farm, during harvesting, or during transportation, or contamination may have occurred in the supermarket during handling. It is possible that *Salmonella* may have been introduced into the melon flesh from the rind by the physical act of cutting the cantaloupe or direct contact with contaminated rinds. The objectives of this study were to investigate (i) the attachment and survival of *Salmonella* Stanley on cantaloupe surfaces, (ii) the efficacy of chlorine or hydrogen peroxide treatments in inactivating *Salmonella* on cantaloupe surfaces, (iii) the possibility of transfer of *Salmonella* to the melon flesh during rind removal and cutting, and (iv) the ability of *Salmonella* to grow on fresh-cut pieces. *Salmonella* Stanley was implicated in an outbreak of salmonellosis associated with contaminated alfalfa sprouts, and our study was designed to investigate the ability of this strain to survive and grow on melons.

MATERIALS AND METHODS

Bacterial strain, growth conditions, and preparation of microbial inoculum. *Salmonella* Stanley HO558, a clinical isolate from the USDA–ARS–ERRC culture collection, was maintained on brain heart infusion agar (BHIA; Difco Laboratories, Detroit, Mich.) slants held at 4°C. Prior to use, the culture was subjected to two successive transfers by loop inocula to 5 ml brain heart infusion broth (Difco). A third transfer of 0.2 ml was made

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into 20 ml brain heart infusion broth incubated at 35°C for 18 h without shaking. Cells were harvested by centrifugation (10,000 × *g*, 10 min) at 4°C. The cell pellets were washed twice in salt peptone (0.85% NaCl, 0.05% Bacto-peptone; Difco). The cell pellets were suspended in salt peptone, and the suspension was transferred to 3 liters of peptone water (0.1%). This suspension served as the inoculum for the test cantaloupes. The final concentration of *Salmonella* Stanley in the inoculum was 10^8 CFU/ml as determined by plating serial dilutions on SS agar (Difco) followed by incubation at 35°C for 24 to 48 h.

Inoculation of cantaloupes. Cantaloupes weighing between 1,461.5 and 1,948.1 g purchased from a local supermarket were placed on a benchtop for 18 to 20 h to allow the melons to come to room temperature (~22°C) before being inoculated. Cantaloupes were submerged in 3 liters of bacterial inoculum and agitated by stirring with a glove-covered hand for 10 min to ensure even inoculation. After inoculation, the cantaloupes were placed inside a biosafety cabinet to dry for 1 h and then stored at 20 or 4°C for up to 6 days before sanitizer treatments were applied.

Wash and sanitizer treatments. Three wash treatments were tested: sterilized tap water, 1,000 ppm chlorine, and 5% hydrogen peroxide. The chlorine solutions (1,000 ppm) were prepared by diluting Clorox, a commercial bleach containing 5.25% NaOCl, in sterile distilled tap water and adjusting the pH to 6.4 ± 0.1 by adding citric acid. Free chlorine in the solution was determined with a chlorine test kit (Hach Co., Ames, Iowa) that has been approved by the U.S. Environmental Protection Agency. Solutions of 5% hydrogen peroxide were prepared from a 30% stock solution (Fisher Scientific, Suwanee, Ga.) by dilution with sterilized distilled water.

Inoculated cantaloupes stored at 20 or 4°C for 0, 24, 72, 120, or 144 h were randomly selected and treated as follows: unwashed (control); washed in sterilized tap water; washed in 1,000 ppm chlorine; or washed in 5% hydrogen peroxide solution. All washing treatments were performed by submerging the melons under the surface of the wash solution then manually rotating the melons to ensure complete coverage and contact of surfaces with solution. Washing treatments were for 5 min, and the washed melons were placed on crystallizing dishes inside a biosafety cabinet to dry for 1 h.

Enumeration of attached bacteria. To enumerate attached, viable *Salmonella,* all inoculated cantaloupes were first washed with 3 liters sterile tap water for 1 min to remove unattached cells. Plugs (n = 70) of cantaloupe rind (2.2 cm) weighing approximately 25 g total were cut with a sterile stainless steel cork borer and blended (Waring commercial blender, speed level 5, 1 min) in 75 ml of 0.1% peptone water. Viable counts were obtained by plating (0.1 ml) in duplicate on SS agar (Difco) followed by incubation at 35°C for 48 h. For comparison, a pure culture of *Salmonella* Stanley (HO558) was plated on SS agar (Difco), incubated as mentioned above, and run parallel with the samples. Selected black or black-centered colonies isolated from the SS agar plates were confirmed to be *Salmonella* according to the Food and Drug Administration (FDA) *Bacteriological Analytical Manual (1)* following conventional biochemical methods.

Transfer of *Salmonella* Stanley from the rind to the flesh during cutting. Inoculated cantaloupes, with or without prior washing treatments, were cut into four sections using a sterile knife. Each section was further cut, and the rinds were carefully removed. The interior flesh was cut into ~3-cm cubes, and 100 g of the cubes was placed in a Stomacher bag along with 200 ml 0.1% peptone water Difco nutrient broth and pummeled for 30 s in a Stomacher model 400 (Dynatech Laboratories, Alexandria, Va.) at medium speed. Viable counts were obtained by plating (0.1 ml) in duplicate on SS agar (Difco) as stated above. Fresh-cut pieces from chlorine- or hydrogen peroxide-treated whole melon were analyzed for the presence of *Salmonella* through preenrichment steps. Instead of 0.1% peptone water, the interior flesh was pummeled for 30 s in a Stomacher model 400 with 200 ml Difco nutrient broth. During the preenrichment step, the broth was incubated at 35°C for 18 to 22 h. For selective enrichment, a 1-ml aliquot of the preenriched sample was added to 9 ml of tetrathionate broth (Difco) and incubated at 35°C for 24 h. Enrichment cultures were plated on SS agar (0.1 ml/plate) and incubated as stated. Biochemical confirmation of identity was performed on the isolates as stated above.

Growth of *Salmonella* Stanley on fresh-cut cantaloupe tissues. In experiments designed to study growth of *Salmonella* Stanley HO558 on fresh-cut cubes, melon flesh from uninoculated cantaloupes surface sanitized by dipping in chlorine or hydrogen peroxide solution for 5 min was cut into ~3-cm cubes using a sterilized stainless steel knife. The fresh-cut cubes (100 g) were inoculated by dipping in an inoculum of *Salmonella* Stanley containing 10^4 or 10^6 CFU/ml for 1 min to achieve an initial population of 10^2 to 10^3 CFU/g on the cubes. The inoculated fresh-cut cubes were placed in sterile Stomacher bags and incubated at 4, 8, or 20°C. Growth was monitored periodically by stomaching cubes as stated above, preparing decimal serial dilutions with 0.1% peptone water where appropriate before plating on SS agar, and confirming selected colonies as *Salmonella* as described above.

Statistical analyses. Three replicates per treatment for each experiment were conducted, and experiments were done twice. Means of replicate data from each treatment were subjected to the SAS (Statistical Analysis Systems Institute, Cary, N.C.) for analysis of variance and to the Bonferroni Least Significant Difference method to determine significant differences within a given treatment over various sampling times. Each value presented represents a mean of average data from two experiments ± the standard error (SE).

RESULTS

Effect of sanitizer treatments. The initial level of attached *Salmonella* Stanley HO558 on the surface of inoculated cantaloupe surfaces was 3.8 $\log_{10}$ CFU/cm^2 and did not significantly ($P < 0.05$) change during storage at 4°C (Fig. 1) or 20°C (Fig. 2) for up to 144 h. Results of sanitizer treatments on the attached, viable population of *Salmonella* Stanley HO558 on cantaloupes stored at 4°C for up to 6 days are shown in Figure 1. Washing with water alone was not effective in removing surface-adherent *Salmonella* Stanley HO558. Washing inoculated cantaloupe stored at 4°C for 0 or 24 h in chlorine (1,000 ppm) for 5 min resulted in a 3.4-$\log_{10}$ CFU/cm^2 reduction in attached *Salmonella* Stanley HO558. The efficacy of the chlorine treatment was less for the longer storage periods. A 5% hydrogen peroxide wash caused a 3.2-$\log_{10}$ CFU/cm^2 reduction at 0 time, which decreased to 1.6 $\log_{10}$ CFU/cm^2 at 24 h and to 0.8- to 0.9-$\log_{10}$ CFU/cm^2 reductions thereafter.

The antimicrobial efficacy of sanitizer treatments applied to inoculated cantaloupes stored at 20°C for up to 144 h is shown in Figure 2. Like the cantaloupes stored at 4°C, washing with water did not reduce the attached, viable *Sal-*

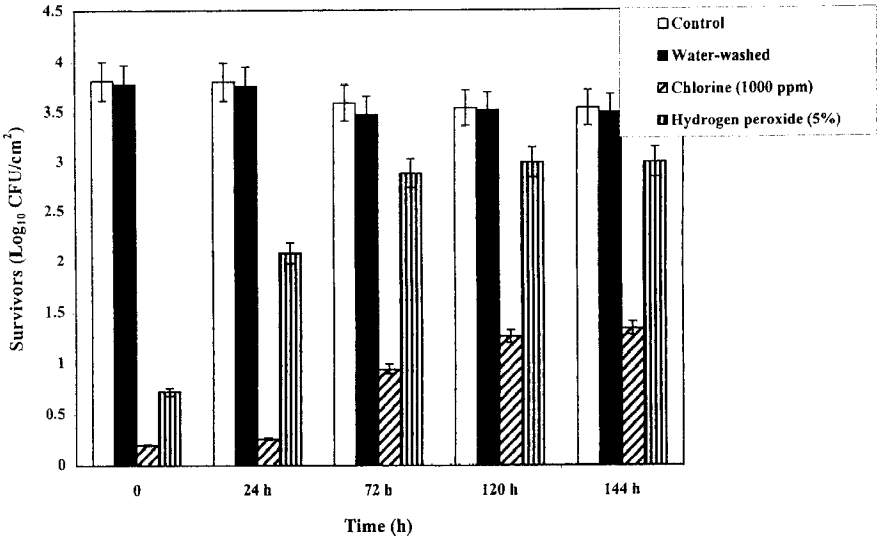

FIGURE 1. *Effect of sanitizer treatments on viability of attached* Salmonella *Stanley HO558 on the surface of cantaloupes stored at 4°C for up to 6 days after inoculation. Values represent mean ± SE of values from three separate experiments.*

monella population on the cantaloupe surfaces. Treatments with chlorine or hydrogen peroxide immediately after inoculation resulted in an approximate 3-log reduction of *Salmonella.* Once again, the efficacy of both treatments declined as the interval between inoculation and the sanitizer treatment increased. Treatment with chlorine, but not hydrogen peroxide, was more effective on melons stored at 4°C than at 20°C, possibly reflecting formation of chlorine-resistant biofilms at the higher temperature.

Transfer of *Salmonella* from the melon surface to fresh-cut cubes. Results of experiments designed to assess the transfer of *Salmonella* Stanley HO558 on cantaloupe rinds to fresh-cut cubes during preparation are shown in Tables 1 and 2. Fresh-cut cubes prepared from water-washed inoculated cantaloupes that had been stored at 4°C for up to 5 days before treatment and cutting were all *Salmonella* positive (Table 1). Recovery of *Salmonella* in fresh-cut cubes prepared from cantaloupes inoculated and washed with chlorine or hydrogen peroxide within 24 h of inoculation was below the detectable limit. However, pathogens transferred to the fresh-cut cubes prepared from inoculated cantaloupes were detected from cubes stored at 4°C from 3 to 5 days prior to chlorine or hydrogen peroxide treatment. Analysis of inoculated cantaloupes stored at 20°C prior to sanitizer treatments showed the same trend (Table 2) as seen in samples stored at 4°C.

The results of a study designed to monitor bacterial growth in fresh-cut cubes after contamination during cutting and subsequent storage at 4, 8, or 20°C for up to 14 days are shown in Table 3. *Salmonella* was detectable in fresh-cut cubes stored at 20°C for 2 days from the chlorine-treated cantaloupes. The study was terminated on day 4 due to the presence of slime, odor, and mold. In samples stored at 4°C for up to 14 days, recovery and numbers of *Salmonella* were below the detectable limit for up to 6 days and for 2 days in samples stored at 8°C.

The ability of fresh-cut cantaloupe cubes directly inoculated with *Salmonella* and stored at different temperatures to support bacterial growth was also determined (Fig. 3). When the initial inoculum on the fresh-cut cubes stored at 4 or 8°C was 10^2 CFU/g, the population of *Salmonella* remained the same throughout the storage period (Fig. 3A). However, growth in fresh-cut cubes stored at 20 or 30°C was evident by 6 h and reached 4 to 6 log CFU/g (a 2.0- to 4.0-log CFU/g increase) at the end of storage, respectively. Increasing the initial inoculum on the fresh-cut cubes

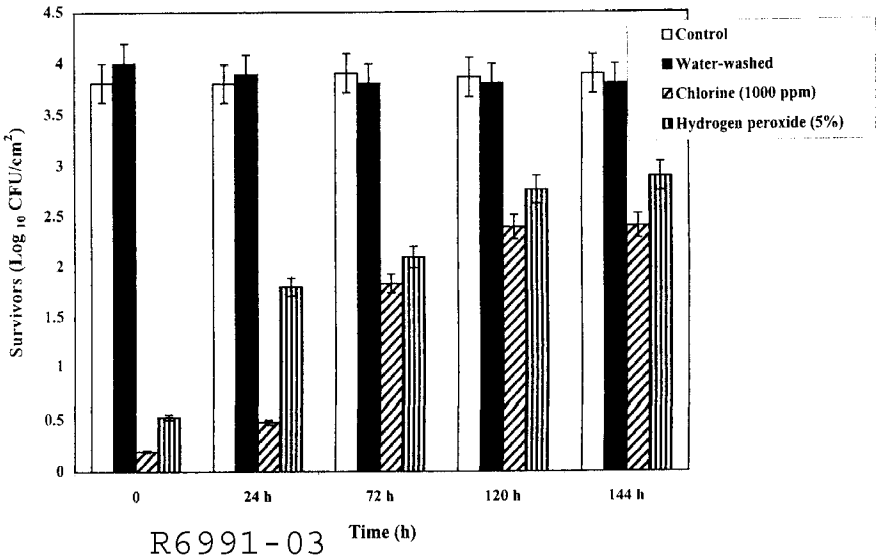

FIGURE 2. *Effect of sanitizer treatments on viability of attached* Salmonella *Stanley HO558 on the surface of cantaloupes stored at 20°C for up to 6 days after inoculation. Values represent mean ± SE of values from three separate experiments.*

TABLE 1. *Recovery of* Salmonella *Stanley in fresh-cut cubes prepared from surface-inoculated cantaloupes stored at 4°C for up to 5 days followed by sanitizer treatments*[a]

	Log_{10} CFU/g			
Days	Control	Water-washed	Chlorine	Hydrogen peroxide
0	0.21 ± 0.02	0.21 ± 0.01	BD[b]	BD
1	0.23 ± 0.01	0.20 ± 0.02	BD	BD
3	0.22 ± 0.02	0.20 ± 0.02	0.12 ± 0.02	0.16 ± 0.02
5	0.22 ± 0.01	0.23 ± 0.01	0.18 ± 0.02	0.20 ± 0.01

[a] Values are means ± SE of duplicate determination from three trials.

[b] BD, below detection (<0.1 CFU/g) at 2- or 24-h postinoculation and washing treatments.

TABLE 2. *Recovery of* Salmonella *Stanley in fresh-cut cubes prepared from surface-inoculated cantaloupes stored at 20°C for up to 5 days followed by sanitizer treatments*[a]

	Log_{10} CFU/g			
Days	Control	Water-washed	Chlorine	Hydrogen peroxide
0	0.22 ± 0.01	0.20 ± 0.02	BD[b]	BD
1	0.21 ± 0.02	0.20 ± 0.01	BD	BD
3	0.24 ± 0.01	0.21 ± 0.02	0.14 ± 0.01	0.18 ± 0.01
5	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.14 ± 0.02

[a] Values are means ± SE of duplicate determination from three trials.

[b] BD, below detection (<0.1 CFU/g) at 2- or 24-h postinoculation and washing treatments.

to 10^3 CFU/g did not cause any significant ($P < 0.05$) changes to the population throughout storage at 4°C (Fig. 3B). However, a 0.5-log CFU/g increase in population was observed in samples stored at 8°C for 6 h but remained the same thereafter. In samples stored at 20 or 30°C, growth was evident by 4 h and increased by 3.8 or 5.2 log_{10} CFU/g at the end of storage (10 h), respectively. In general, there was a lag time of 4 h before observing *Salmonella* growth in fresh-cut samples at all temperatures when the initial population was 10^2 CFU/g. An increase in initial population to 10^3 CFU/g decreased the lag time by 2 h in fresh-cut samples stored above 8°C.

DISCUSSION

Some melons are treated with antimicrobials or wax to retard invasion by spoilage organisms by the processors *(18)*. However, some producers are field packing melons for direct shipment; therefore, treatments intended to retard spoilage are not applied. At the retail level or at food establishments, melons are usually washed using only potable water, and the fresh-cut pieces are prepared using clean and sanitized utensils. Thus, fresh-cut melon may not be adequately protected from contamination.

In our studies, washing of laboratory-inoculated whole cantaloupes in chlorinated (1,000 ppm) water within 24 h after inoculation reduced the population of attached *Salmonella* Stanley on the cantaloupe surface and the possibility of transfer during fresh-cut preparation. Fresh-cut cubes prepared from water-washed cantaloupes were *Salmonella* positive, suggesting the need for a better washing treatment. *Salmonella* Miami and *Salmonella* Bareilly were responsible for two salmonellosis outbreaks associated with precut wrapped watermelon, according to Gayler et al. *(8)*. They showed that the interior watermelon tissue could be contaminated if *Salmonella* was present either on the rind of the watermelon or on the knife used for slicing. They failed to report the initial inoculum size or the final population attained on the watermelon flesh. Similarly, transfer of *Salmonella* from the surface of tomatoes to the interior during cutting was reported by Lin and Wei *(13)*. Their data suggested that the rate of bacterial transfer is dependent on inoculum size on the stem scar. In our study, we observed less than the detectable limit when the inoculum size was reduced by the sanitizer treatment, compared to fresh-cut cubes prepared from inoculated cantaloupes given no treatments or those stored 3 to 5 days before treatments. Golden et al. *(10)* reported a 5-log CFU/g increase of *Salmonella* spp. on fresh-cut cantaloupe from an initial population of 10^2 CFU/g after incubation at 23°C for 24 h.

Salmonella is among the most frequently reported causes of foodborne outbreaks of gastroenteritis in the United States, and recently, salmonellosis associated with consumption of contaminated cantaloupe has been reported *(8)*. The pathogen may have been introduced into the flesh from the rind by cutting or contact of cut pieces with contaminated rinds. Consumption of contaminated watermelon has also been implicated in outbreak of salmonellosis *(5, 7, 8)*. We do not know if contamination occurs through direct contact with fecal matter or contaminated soils or by contaminated irrigation water. Contamination has been attributed to growing produce in soil contaminated or irrigated with sewage effluents *(11, 14, 20)*. Our data suggest that an outbreak of disease might result from eating contaminated fresh-cut melon prepared from improperly washed or surface-sanitized cantaloupe stored at 4 or 20°C for more than 3 days. These results indicate that populations of the pathogen attached on the melon surface for more than 3 days were difficult to reduce using chlorine or hydrogen

TABLE 3. *Recovery of* Salmonella *Stanley from fresh-cut cubes prepared from chlorine-washed inoculated cantaloupes stored at different temperatures for 14 days*[a]

Storage temperature (°C)	Day(s)[b]							
	0	2	4	6	8	10	12	14
4	—	—	—	—	++	++	++	++
8	—	—	++	++	++	++	++	++
20	—	++	++					

[a] Fresh-cut pieces represent 24-h postinoculation and treatment as seen in Tables 1 and 2. Results are based on three trials and duplicate determinations.

[b] ++, *Salmonella* positive; —, *Salmonella* negative.

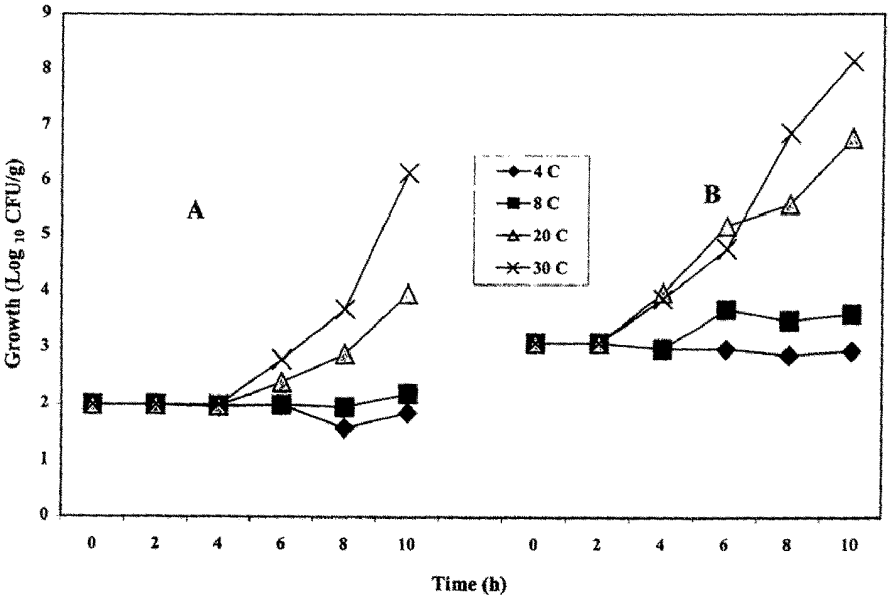

FIGURE 3. *Survival and growth of* Salmonella *Stanley HO558 on fresh-cut cantaloupes cubes directly inoculated at two levels (A = 10^2 CFU/g, B = 10^3 CFU/g) and stored at different temperatures for 10 h.*

peroxide treatment. Therefore, fresh-cut preparation with such melons might result in contaminating the internal flesh used for fresh-cut purposes. The levels of *Salmonella* recovered from fresh-cut melon prepared from water-washed inoculated cantaloupes were less than 0.25 $\log_{10}$ CFU/g, whereas the population of those artificially inoculated directly on fresh-cut melon stored at 4°C did not change throughout storage. However, growth occurred when inoculated (10^3 CFU/g) fresh-cut cubes were stored at 20°C for 4 h. Golden et al. *(10)* reported similar findings for cantaloupes inoculated with *Salmonella* and stored at 23°C for 1 h.

The efficacy of washing treatments on detachment or inactivation of *Salmonella* on cantaloupe surfaces is dependent on the state and location of the organisms on the outer surface of the cantaloupes. The results of this study suggest that the *Salmonella* attached to cantaloupe surfaces can survive at least 6 days. Survivability of *Salmonella* ranged from 10 to 53 days on root crops and from 1 to 40 days on leaf vegetables *(17)*. *Salmonella* Stanley transferred from the rind to the fresh-cut cubes has the potential to grow because of available nutrients if the temperature is favorable. Tamplin *(19)* reported that attention should be directed to cleaning the melons at the time of cutting, using clean and sanitized utensils and surfaces to minimize contamination of the edible portion, and immediately consuming or else holding cut melon pieces at cold temperatures.

In all outbreaks noted so far, all reports mentioned melons, which had been precut and held at unknown temperatures for some period of time at retail prior to being purchased and consumed. The inner flesh of fresh-cut melons is composed mainly of parenchyma cells *(11)* containing sugars, organic acids, and other substances that may support microbial growth as observed in our study. Golden et al. *(10)* reported growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons stored at 23°C. Other investigators *(6)* have reported that interior watermelon tissues support the growth of *Salmonella* spp. Evidence of bacterial internalization of intact cantaloupe has not been reported, and this may be attributed to the protective nature of the cantaloupe rinds until cutting compromises this barrier.

In conclusion, proper refrigeration will control the growth of *Salmonella* on fresh-cut cantaloupes. Melons implicated in outbreaks may have been contaminated during fresh-cut processing and may have been temperature abused for a long period to allow the organism to increase to a level capable of causing illness. We therefore recommend that cantaloupe surfaces be washed with chlorinated water, or an alternative sanitizing agent, and rinsed with water before cutting. Utensils and surfaces in contact with the melons need to be carefully sanitized when preparing fresh-cut melons. The fresh-cut product should be maintained at 4°C to suppress growth. However, because the time of contamination is not generally known and may precede washing by many days, more effective means of decontaminating cantaloupes are needed.

ACKNOWLEDGMENTS

We acknowledge the valuable assistance of Dr. John G. Phillips in statistical analysis of the data and that of Drs. Pina Fratamico and William Fett for their valuable and critical review of this manuscript.

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